## Labels

▪ Ruijie Tang ▪ May 18, 2016 @08:12 AM EDT

1. For MCF tubes:

   P: Plasmid Prep

   D: Digest

   T: Transformation

   L: Ligation

## Transformation

▪ Lydia Goff ▪ May 31, 2016 @10:50 AM EDT

1. x = the number of transformations you are doing
2. Place the SOC medium (from the fridge) in the incubator
3. Also place X plates in the incubator (With the appropriate antibiotic resistance, Chl for complete, Amp for parts)
4. Make an ice-water bath
5. Obtain X MCF tubes and a float
6. Add 50ul of competent cells
7. Add 2ul of the DNA
8. Place it in the ice-water bath for 2 mins
9. While waiting
   - Turn on the sink's hot water
   - Obtain a beaker
   - Add hot water
   - Using a thermometer, adjust water to 42$^{o}$ C (using the hot plate if needed)
10. Place tubes in the incubator for 30 seconds
11. Another 2 mins on ice
12. Add 1ml of warm SOC medium
13. Place each tube in the incubator (37$^{o}$ C) for 1 hour
14. Plating

## Pick colonies

▪ Ruijie Tang ▪ May 18, 2016 @11:05 AM EDT

1. Obtain the green sticks, empty LB tubes, and the plates from the incubator
2. Label the tubes with corresponding plates
3. Spot the colonies (Choose more than one ifavailable)
4. Use the sticks and gently obtain the colonies
5. Swirl around in the LB solution and dispose the stick into the tip trash
6. Put the tubes into the shaking incubator overnight

## Plasmid Prep Procedure

▪ Lydia Goff ▪ Sep 06, 2016 @04:16 PM EDT

1. Transfer 1.5ml bacterial culture to a labelled MCF tube
2. Centrifuge for 30 seconds to pellet bacteria
3. Pour off most of the growth medium. Leave approximately 100ul in the tube
4. Resuspend the pellet by shaking, vortexing, or tapping the tube vigorously
    - Make sure the bacteria are completely resuspended and no clumps remain before continuing
5. Add 100ul TENS to the tube

- TENS procedure (make fresh each time)-To 4.5 ml TE solution add 250 ul 10% SDS and 250 ul 2M NaOH

1. Mix well by inversion for 2 minutes. Do not shake the tube
    - The solution will get viscous
2. Add 150ul sodium acetate and mix well
3. Centrifuge for 3 minutees
4. Transfer the supernatant (liquid) to a clean, lableled MCF tube
5. Add 1ml 75% Ethanol to the tube and mix will by inversion. You may see DNA as faint white strands in the liquid
    - The tube should be nearly full of liquid
    - Sharpie brand pens have an ink that is soluble in Ethanol. Make sure your lables do not get removed accidentally
6. Centrifuge 5 minutes to pellet the DNA
    - Place the tubes in the centrifuge with the hinges out to make finding the pellet easier
7. Pour off the 95% Ethanol
8. Place the tubes upside-down and allow them to dry completely
9. Resuspend the pellet in 25 uL TE
10. Use 10 uL pf the DNA in a restriction digest or 1 uL for PCR

*This procedure does not

## Digest

▪ Lydia Goff ▪ Sep 08, 2016 @03:24 PM EDT

1. obtain the plasmid prep tubes (in incubator), new MCF tubes, enzymes in the freezer

2. For each new tube, prepare the master mix(MM):

    water 15ul

    buffer 2ul

    Upstream enzyme 0.5ul

    Downstream enzyme 0.5ul

Each tube needs 18ul of the MM, in order to get enough MM into each tube. If you have x digest, do x+1 MM. (Ex. If I need to do a digest for one tube, I will prepare 30ul of MM, but still put 18ul into the tube)

3. Label the new tubes with corresponding tubes and change the "P" into a "D"

4. Add 3ul DNA and 15ul MM into each tube

5. Obtain a tube float and put all the tubes into the dry incubator for at least an hour.

## How to make gel

▪ Lydia Goff ▪ May 19, 2016 @03:39 PM EDT

1. Obtain one 125ml conical flask, 1x TAE solution, and agarose

2. With the balance, measure 0.32g of agarose

3. Use a measuring cylinder and measure 40ml of 1x TAE solution

4. Put the measured powder and liquid into the conical flask (make sure no powder is stuck to the wall)

5. Use plastic film and wrap around the opening of the flask, BUT remember to leave a hole. Do not completely seal the flask

6. Microwave the solution for 1 minute

7. Use a glove and transfer the solution from the Microwave to a black top table

8. Find the well for the gel, duct tape, and combs (depends on the number of digest you have, you need one or two combs)

9. Tape the edges of the well and make sure the bottom is sealed. Put the combs in

10. When the microwaved solution is cooled down a bit, add 4ul of ethidium bromide into the flask and swirl

11. Pour the solution into the well and wait til it forms a gel like substance

*12. If you need to store it for future uses, obtain a zip lock bag

*13. Tear off the tape carefully, but leave the combs in

*14. Store them into the refrigerator not the freezer

## Run the gel

▪ Lydia Goff ▪ Jun 21, 2016 @11:23 AM EDT

1. Put 4ul of dyes into each tube of digest, make sure the dye is well mixed
2. obtain the made gel and put it into the machine (clear one on counter in closet)
3. Use 0.25x TAE, and pour it until the gel is totally submerged into the solution
4. Take a paper and record the order of tubes when you put them in
5. For each roll there are eight empty places **The first place of each roll have to be the ladder (4ul)
6. Obtain the total 24ul solution from each tube and add into the gel
7. Close the lid and turn the machine on "high" for approximately 25 minutes
8. Turn off the machine before removing the lid
9. Turn off the backroom light and place the gel on the UV light
10. Turn on the UV light and observe

## Ligation

▪ Lydia Goff ▪ Jun 09, 2016 @09:28 AM EDT

1. Prepare the mix
    - Upstream Part digestion - 2ul
    - Downstream Part digestion - 2ul (replace with water if no Downstream is available, same amount)
    - Destination Plasmid digestion - 2ul
    - 10X T4 DNA Ligase Buffer - 2ul
    - T4 DNA Ligase - 1ul
    - Distilled $H_2O$ - 11ul

1. Incubate in the incubator for 10 mins
2. Heat activate at 80$^0$C for 2 mins (hot plate)

## Making a Plate

▪ Ruijie Tang ▪ May 19, 2016 @04:37 PM EDT

1. Obtain distilled water, agar, and a 500 ml conicial flask (Blue cabinet)
2. According to the proportion on the label of agar, make a 300 ml solution
3. Use a magnetic stirring bar, tilt the flask and push the bar into the flask
4. Put the flask on the hot plate, and turn on the stirring button to high
5. Seal the opening with aluminum foil and autoclave it with standard procedure
6. Pull it down and let it cool until solidified
7. When you need to use it, put it on the hot plate and heat it up for about 10 mins
8. Then, turn on the stirring botton
9. ALWAYS keep an eye on the flask. When all the solid turns into liquid, stop heating the flask
10. Transfer the flask to a black surface and when it is warm, add appropriate antibiotics in it (300 ml of agar needs 300 ul of antibiotics)
11. Normally, 300 ml can pour up to 10 plates, so obtain 12 in case
12. Pour only a thin layer of agar into each plates
13. Put corresponding color stripes onto the side of the plates

## Making different arabinose concentration

▪ Lydia Goff ▪ May 19, 2016 @03:44 PM EDT

Normally we do 0%, 0.001%, 0.01%, 0.1%, 1%, 2%, 4%

1. The arabinose concentration is in the freezer

2. Use the LB tubes and add the accurate amount of arabinose in. Remember no matter what the concentration of arabinose is, you cannot add more than 200ul of arabinose solution into the LB. Therefore, if needed switch to use 20% arabinose instead of the 0.2% arabinose with the concentration needs more than 200ul of 0.2% arabinose

3. Add the cells (from Pick colonies tubes, ask Ms. Byford for amount) into the different concentration

4. Put them into the shaking incubator overnight

## Autoclave

▪ Ruijie Tang ▪ May 19, 2016 @04:39 PM EDT

1. Put everything you want to sterile in the big pressure cooker
2. close the lid
3. Turn the hot plate to 10
4. Until you hear the "ziiiii" sound, time for 15 mins
5. When done, put it in the hood and let it cool

## Modified Competent Cells

▪ Lydia Goff ▪ May 31, 2016 @09:44 AM EDT

1. Start liquid culture of DH5 α cells (single colony) from plate
    a. Grow overnight
2. Get_pre-chilled MCF tubes from fridge
3. Prepare ice-water bucket
4. Evenly transfer DH5α cells into MCF tubes
5. Centrifuge for 10 mins @ 6000 rpm
    a. Use the smaller clinical centrifuge
6. Prepare bleach container
7. Decant supernatant into bleach
8. Add 400λ of ice cold CCMB80 buffer
    a. Resuspend using the pipet until solution is consistent
    b. Then pipet for another 5 cycles (in and out)
9. Add another 400λ of the buffer
    a. Shake gently by inversion
10. Place in ice bucket for 20 mins
11. Centrifuge again for 10 mins
    a. Same instructions as above
12. Decant supernatant into bleach
13. Resuspend in 175λ of buffer
    a. Same instructions as above
14. Place in ice bucket for 20 mins
15. Label the float and tubes!
    a. Date and initials!
16. Use accordingly or store in large freezer
    a. See "transformation procedure" for more info

## Growth Curve Procedure

▪ Will Hayes ▪ Jun 08, 2016 @10:26 AM EDT

Procedure (for all figures refer to source 1-a)

1. Preparation
    1. Materials
        1. E. coli culture(s)
        2. trypticase soy broth
        3. 50 ml flask(s)
        4. micropipettes
    2. 2 days before experiment

1. Inoculate a 50 ml flask of trypticase soy broth (TSB) medium with E. coli. Incubate overnight at 27°C. This will yield 109 CFU/ml.

3. 1 day before experiment

    1. Use 100ml of the prepared culture to inoculate 250 ml of TSB (in a 500 ml flask). Mix thoroughly and remove 5 ml and refrigerate immediately. This is T = 0 and will yield approximately 5 ¥ 105 CFU/ml. Place the flask of E. coli in a 37°C shaking incubator. Remove 5 ml aliquots of culture every hour up to 8 hours. Store each aliquot at 4°C. These cultures should be designated T0 through T8.

2. Procedure

    1. Materials

        1. 1 ml aliquots of E. coli broth cultures (or other bacterium)
        2. 0.9 ml sterile water dilution tubes in microfuge tubes
        3. micropipettes
        4. poured Petri plates with trypticase soy agar
        5. ice
        6. glass hockey stick spreader
        7. vortex mixer
        8. gas burner
        9. ethyl alcohol for flame sterilization

    2. Make a 10-fold dilution series
    3. For one dilution, transfer 0.1 ml of suspension to each plate. After inoculating all replicate plates in one dilution, go to step 4. Repeat for next two dilutions.
    4. For each plate, sterilize a glass hockey stick spreader in a flame after dipping it in ethanol. Let the spreader cool briefly. Go to step 5.
    5. Briefly touch the spreader to the agar of an inoculated plate to cool, away from the inoculum. Then, spread the inoculum by moving the spreader in an arc on the surface of the agar while rotating the plate. Continue until the inoculum has been absorbed into the agar. Repeat 4 and 5 for the other replicates. Then, go to step 6.
    6. Repeat steps 3, 4, and 5 for each dilution. When done, let the agar dry for a few minutes, tape the plates together, and incubate them upside down for one week.
    7. Set up a series of dilution tubes to obtain dilutions of 10-1 through 10-7 of the E. coli cultures. Microfuge tubes are convenient to do this (see Figure 1-3). Each dilution tube will have 900 ml of dilution fluid (sterile saline). A dilution series will be needed for each E. coli culture (T0 thru T8).
    8. Begin dilutions by adding 100ml of E. coli from the tube labeled T0 which is the initial E. coli culture to tube A.Tube A is the 10-1 dilution of T0.
    9. Vortex the 10-1 tube for 5 seconds.
    10. Follow this by subsequently adding 100 ml of Tube A to the next tube of saline (Tube B). Tube B is a 10-2 dilution of T0. Repeat until completing the dilution series, referring to Table 1-2 to see how far you will need to make dilutions for each E. coli culture. Remember to vortex each tube prior to transfer. It is also important to use a new pipette tip for each transfer.
    11. Repeat dilutions for T1 through T8 or for whatever samples were assigned to you. Again refer to Table 1-2 to see how far you need to make your dilutions.
    12. Plate according to the regiment specified in Table 1-2.
    13. Label plates with the dilution and volume to be added to the plate. Make sure the label contains the time point plated (T1 thru T8) identification. Use triplicate plates for each dilution.
    14. Pipette 100ml from each of the three dilutions to be plated. Add 100ml of each dilution tube to be plated by pipetting the amount to the center of the agar plate (Figure 1-3).
    15. Immediately spread the aliquot by utilizing a flame sterilized “L” shaped glass rod. If the aliquot is not spread immediately, it will sorb in situ in the plate resulting in bacterial overgrowth at the spot of initial inoculation.
    16. Repeat the plating for each dilution series for T1 through T8 cultures. Remember to sterilize the rod in between plates and especially between different dilutions.
    17. Once plates have dried for a few minutes, invert and place in 37°C incubator overnight. Following this, store plates in refrigerator.

3. Examining the plates

    1. Examine plates for uniformity of colonies and lack of contamination (see Figure 1-4).
    2. For each culture (T0 through T8), count triplicate plates at one dilution that contains between 30 and 300 colonies.
    3. Calculate the number of cells per ml of original culture for T0 through T8 cultures.
    4. For example, the number of colonies resulting from a 10-4 dilution is 30, 28, and 32.

        1. Mean number of colonies = 30 colonies
        2. These arose from 0.1 ml of a 10^-4 dilution
        3. Number of colonies per ml = (30*10^-4)/0.1

5. Plot log10 CFU/ml versus time (hours).
6. From the graph, identify the exponential phase of growth. Using two time points within the exponential phase of growth and corresponding cell numbers, calculate the mean generation time.

Sources

1. Dilution and Plating of Bacteria and Growth Curves
    1. http://booksite.elsevier.com/samplechapters/9780125506564/Sample_Chapter/02~Experiment_1.pdf

## Growth Curves

▪ Lydia Goff ▪ Jun 30, 2016 @11:23 AM EDT

1.